D7

~~13. (Twice Amended) An antibody as claimed in Claim 25, wherein the constant domains of the antibody have substantially the same amino acid sequence as the therapeutic antibody constant regions.~~

D8

~~14. (Twice Amended) A fragment of an antibody according to Claim 25, which fragment retains tolerance-inducing capability of the antibody.~~

D9

~~16. (Twice Amended) An antibody or fragment as claimed in Claim 25, which is monovalent.~~

D10

~~17. (Twice Amended) An antibody or fragment as claimed in Claim 25, for inducing tolerance to the therapeutic antibody in a patient.~~

REMARKS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The application has been amended to include an Abstract. No new matter has been added.

The specification has been amended to make reference to the PCT from which the present case derives.

Applicants do not understand Campath-1 to be a trademark. Thus no revision in that regard is believed to be necessary.

Sequence identifiers were introduced via the Amendment filed November 9, 1999.

Claim 1 has been revised and includes features of now cancelled claims 3-5. The remaining claims have been revised to correct dependency.

Claims 1-17 and 22 stand rejected under 35 USC 112, first paragraph, as allegedly being non-enabled (paragraphs 9 and 10 of the Action). The rejections are traversed.

In response to the Examiner comments that "[I]n vitro and animal models have not correlated well with in vivo clinical trial results in patients", Applicants have the following comments.

Immunology has worked on the fact that there are many rules common across species, and Applicants' own work on CAMPATH-1 antibodies has been dependent on such in-vitro and animal studies in its long history.

For example rat IgG2b antibodies were better than other subclasses for killing lymphocytes in-vivo CAMPATH-1H and IgG2b antibodies were the best at ADCC and complement fixation in-vitro and were also effective in-vivo.

CAMPATH-1H antibodies kill lymphocytes very effectively in transgenic mouse models, just as in the human.

When it comes to tolerance the Examiner quotes a chapter by Auchincloss based on efforts to achieve transplantation tolerance in humans. The Examiner notes that although one can achieve tolerance in rodents it is harder to do so in humans. Respectfully, the Examiner trivializes the issue as indeed CAMPATH-1H is proving one of the agents that is close to achieving clinical tolerance (see Calne et al. Lancet). A major problem is that one cannot try new transplant tolerance strategies in humans while removing the patient from treatment with previously recognized immunosuppressants, such as cyclosporine A and steroids. Recent primate experiments show that these drugs can interfere with tolerance, and so such studies are likely to be flawed anyway. When tolerance is achieved in the human, it will almost certainly be based on principles common to other species, especially rodents.

Applicants' work here does not deal with transplantation tolerance but with a much simpler issue, that is, tolerance to immunoglobulins, where there are far less antigenic differences than in the transplant situation. High doses of non-binding-immunoglobulin would

be very likely to induce tolerance in Applicants' view. The study has not been done of course, because with the advent of humanization technologies, there has been no reason or principal to offer immunologically potentially immunogenic foreign antibodies to a patient. This work in rodents predicts that this can now be done.

Again, Applicants remind the Examiner that there are many principles of immunology that extend from rodents to humans and Applicants are not clear as to why the Examiner elects to dwell on the ones that apparently do not. Examples are humoral immunity, antibodies, cellular immunity, cytotoxic T-cells, ADCC, eosinophil, neiutrophil and macrophage contributions, Th1 and Th2 functions, delayed hypersensitivity, mouse models of immune deficiency and autoimmunity, and inflammatory bowel disease.

In the same vein, one of the robust principles of tolerance research that is widely acknowledged is high dose tolerance induction to monomeric foreign immunoglobulins, and Applicants cannot see, therefore, why the Examiner draws on dissimilarities between species in making the rejection.

In addition, Applicants provide the following details of a number of techniques which would have been well known to the skilled person at the date of the present

application as a means of obtaining, without undue burden, the modified therapeutic antibodies according to the present invention:

Producing a non-binding antibody mutant

1. Computer-generated molecular models can be used to predict the structure of the antibody variable domain and in particular, the combining site for antigen (ACS). These models can be sufficiently accurate to predict CDR loop conformations and for outlining the binding site. In two recent publications, computer-generated models of therapeutic antibodies built from sequence data alone were shown to be very close to the actual X-ray crystal structures of the antibodies [J. Bajorath & S. Sheriff, Proteins 24, 152 (1996)]. Modelling the loop conformations has become routine for L1, L2, L3, H1 and H2 [I.M. Tomlinson et al, EMBO J. 14, 4628 (1995)].

To produce a non-binding antibody mutant, specific residues can then be chosen for mutagenesis and functional screening in a rapid (e.g. M13 vector) expression system. One would choose residues on the basis of accessibility (e.g. possessing a large solvent-accessible surface as for the Lys residues in H2 of CAMPATH-1H), position within the

loop (central positions would have a greater chance of interacting with antigen than those flanking the framework residues), or residue character (large polar or charged residues such as Arg, Lys Asp, or Tyr in the ACS may interact directly with antigen). Site-specific single mutations of an antigen-binding residue to Ala, a small non-polar residue, would probably cause significant disruption to binding with minimal structural change to the loop. Certain residues would not be changed as they are involved in maintaining loop structures [C. Chothia et al, J. Mol. Biol. 227, 799 (1992)].

2. "CDR-swapping" can be used as a rough guide to determine which of the 6 loops hold key antigen-binding residues. Many antibodies use only 4 or 5 of their 6 CDR loops to bind antigen [I.A. Wilson & R.L. Stanfield Curr. Opin. Struct. Biol. 3, 113 (1993)]. Using the method of "CDR-swapping", site-specific mutagenesis (to make minimal mutants) can be confined to relevant (antigen-binding) CDR loops. Six Fab mutants are created, each containing one irrelevant CDR loop derived from the VL or VH sequences that provided framework residues during humanization of the "wild-type" therapeutic antibody [See L.K Gilliland paper mentioned in present application]. These mutants are

assessed for their ability to bind antigen. Fab mutants with irrelevant sequence in a CDR loop that have the same binding characteristics as wild-type antibody demonstrate that that particular loop sequence is not needed for antigen-binding.

3. Alanine scanning. Non-binding antibodies with preserved structure should be obtainable even without a molecular model. Each CDR residue could be mutated in turn to Ala, with the exception of residues that are known to preserve loop structure [C. Chothia et al, J. Mol. Biol. 227, 799 (1992)] which would remain unchanged. Although initially this may require significant labor, PCR-based technology and automated sequencing would allow rapid assembly and verification of constructs. However, significant effort may be required to characterize the binding of each mutant to antigen and this approach might be better suited to industry. The advantage of this approach is that key binding residues will be identified functionally and may be combined to obtain the best possible non-binding mutant. If unique mutations lower the binding affinity but do not destroy binding of the antibody, then a second round of mutagenisis could be carried out to combine 2 of the mutations into a single L- or H-chain, where the mutations

both occur in the VL CDR residues or in the VH CDR residues, respectively. Alternatively, a single mutation in a L-chain could be combined with a single mutation in a H-chain in the expression vector(s).

4. Computer-generated molecular models combined with functional data.

Likely the most sensible approach would be to build a reasonable model of the antibody Fv from VL and VH sequence, while concurrently deriving CDR swapped constructs for functional studies. The model would show residues that are likely to be involved in antigen-binding and would give the additional benefit of a predicted structure so that changes that may alter antibody structure could be avoided (one can test these changes on the model). CDR-swapping would identify which CDR loops are functionally involved in binding antigen. Mutagenesis might then be commenced on a more "general" level such that 2 or 3 residues per loop in Ag-binding CDR could be changed at a time. In constructs where binding is eliminated, a more "specific" round of mutagenesis could be carried out using single residue changes at those key positions. In this way, one could anticipate obtaining a non-binding antibody with

a minimally mutated structure in a relatively short period of time.

Thus, it is submitted, that at the date of the present invention, the skilled person, armed with the above known techniques and the teaching of the present application, would have readily been able to practice the invention in a manner commensurate with the claims, and would have had stong guidance from the knowledge and techniques commonly available at the time as to how to successfully modify therapeutic antibodies to remove binding to an extent sufficient to enable the thus modified antibodies induce tolerance. In short, the present invention is based on the finding that non-mixed molecule therapeutic antibodies which are modified to considerably reduce binding affinity can be used to induce tolerance to therapeutic antibodics. The invention represents a novel and inventive advance over the cited art. To require restriction of the invention to specific structural features would constitute an undue limitation. That many possibilities within the scope of the claim are possible is true, however, given the teaching and premise of the invention and the techniques available at the date of the invention, the embodiment of these possibilities is something that would have been within the ambit of the skilled person without the application of

inventive ability and without undue burden.

Reconsideration is thus requested.

Claims 1-17 and 22 stand rejected under 35 USC 112, first paragraph, as allegedly lacking written description. The rejection is traversed.

The claims as presented relate to modified antibodies and fragments thereof. Experimental details of the production of these modified antibodies are provided in the application as filed in the section entitled Examples on pages 15 to 21. Furthermore, as filed the application provided a written description of the use of the modified antibodies for the stated purpose in a mouse model. Correlation between the mouse model and human studies is discussed fully in the specification and above. In view of this, Applicants contend that the description of the present application is sufficiently detailed to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. Reconsideration is requested.

Claim 10 appears to stand rejected under 35 USC 112, first paragraph. The rejection is traversed.

The antibody of the claim has described in detail (including sequence) by Reichmann et al, Nature 332:323

(1988). Nothing more should be required and reconsideration is requested.

Claims 1-17 and 22 stand rejected under 35 USC 112, second paragraph, as allegedly being indefinite. The rejection is traversed.

The dependant claims are consistent with claim 1 in that none of the dependant claims describes mixed molecules having a V or L chain from a therapeutic antibody combined with a V or L chain from an unrelated antibody.

The use of the term Campath-1 antibody as a designation is not indefinite, rather it would be well known to a person skilled in this field. This should be evidenced by the fact that three of the four cited documents use this reference without any further elaboration.

As indicated above, above, Campath-1 is not understood by Applicants to be a trademark.

The word "molecule" has been deleted from claim 1.

In the context used, the term "substantially" is not believed to render the claims indefinite.

As regards "immunological tolerance", Applicants submit that the phrase would be understood to mean a pre-treatment with a given protein or molecule, irrespective of mechanism, which results in diminished capacity to respond

to that or a further protein or molecule, i.e. mutant/wild type, without affecting response to unrelated antigens.

Reconsideration is requested.

The claims stand rejected under 23 USC 102 over Waldmann et al, Crowe et al, Carter et al or Isaacs et al. Reconsideration is requested in view of the following comments offered by Applicants.

Applicants submit that Waldmann et al describes an antibody which binds effectively with the Campath-1 antigen, and which has at least one CDR of rat origin, which is combined, with a range of different foreign variable domain framework regions including framework regions of human origin. In contrast with the antibodies of the present invention, it is Applicants' view that none of the antibodies disclosed by Waldmann are modified to include an alteration in at least one of the CDR's to produce an antibody having an affinity for antigen which is reduced to less than 50% of the affinity of a therapeutic (unmodified) antibody for the same antigen. In fact, Applicants believe Waldmann is more concerned with providing antibodies which bind effectively with the Campath-1 antigen, which is complete distinction to the teaching of the present invention.

Applicants further submit that Crowe et al describes a method of producing chimeric humanized antibodies in which the CDR of a first antibody is spliced between the framework regions of a second antibody. Applicants contend that it is specifically pointed out on column 8, lines 50 to 54, that the process of the invention is carried out in such a way that the resulting chimeric antibody retains the antigen binding capability of the non-human antibody from which the CDR region(s) is/are derived. Thus, Applicants believe that it is clear that none of the antibodies disclosed in Crowe have an affinity for antigen which is reduced to less than 50% of the affinity of the therapeutic antibody for the antigen. In addition, Applicants believe that Crowe does not disclose antibodies which are modified, wherein the modification comprises an alteration in at least one of the CDR's - rather, in Applicants' view, Crowe teaches chimaeric antibodies whereby the imported CDR is unmodified and unaltered.

Applicants also submit that Carter et al discloses the preparation of humanized antibodies and fragments using recombinant strategies. Applicants direct attention to the definitions section of the specification, column 8, lines 50 to 54, which reads "A humanised antibody for the purposes herein is an immunoglobulin amino acid sequence

variant or fragment thereof which is capable of binding to a predetermined antigen...". From this, Applicants contend it is clear that the purpose of the teaching of Carter is the production of antibodies which bind antigen, which is in contrast to the purpose of the present invention which is the provision of antibodies which have considerably reduced affinity for antigen.

Applicants additionally submit that Isaacs et al discloses the use of helpfulness as a strategy for avoiding anti-globulin responses to therapeutic monoclonal antibodies. This document has already been considered during International Preliminary Examination of the current application wherein the claims as on file during the International Phase were considered novel and inventive over the teaching of this document. Specifically concerning novelty, Applicants point out that the view of the preliminary Examiner was that as the antibodies disclosed in D1 comprised mixed molecules (having a H or L chain of a therapeutic antibody paired with a H or L chain from an unrelated antibody), the disclaimer to mixed molecule antibodies in the present claims was sufficient to establish novelty.

Thus, Applicants submit that claim 1 as amended is novel over all the cited documents. Applicants note that

the documents dealing with humanization of antibodies (Waldmann, Crowe and Carter) mention some antibody products which have a reduced affinity for antigen compared with therapeutic antibody. However, none of the documents are believed by Applicants to provide an enabling disclosure of antibody mutants (wherein the mutations occur in the or each CDR) which have less than 50% affinity for antigen compared to a corresponding therapeutic antibody. Accordingly, reconsideration is requested.

Claims 1-17 and 22 stand rejected under 35 USC 103 as allegedly being obvious over Waldmann et al or Crowe or Carter in view of Isaacs. Reconsideration is requested in view of the following comments offered by Applicants.

Applicants submit that none of the documents dealing with humanized antibodies (Waldmann, Crowe or Carter) deal with the issue of inducing tolerance in an individual. Applicants further submit that none of these documents teachs modification of therapeutic antibodies to effect removal of binding ability. On the contrary all of these documents are seen by Applicants as teaching methods of modifying antibodies such that binding is maintained while the immunogenic response to the modified antibody is lessened by "humanizing" modifications. Thus, taken in isolation, Applicants contend that none of these documents

would motivate the skilled person to make antibodies according to claim 1 for the purpose of the present invention.

Applicants submit the closest of the cited art would appear to be Isaacs which the Examiner contends teaches the use of non-binding variants of therapeutic antibodies to generate therapeutic unresponsiveness to clinical useful antibodies. Applicants understand that a brief superficial consideration of the content of this document may give this impression (see page 303, column 2, first paragraph). However, when the whole content of this paper is considered in detail, Applicants submit that it is apparent that the author clearly teaches against the use of non-cell binding, non-mixed molecule, variants as a means of inducing tolerance. In this regard, it is submitted by Applicants that the discussion section may be the most pertinent - see for example the passage on page 310, column 1, lines 14 to 18 which teaches:

> " There was no special advantage for tolerance induction of using non-cell binding variants of the therapeutic mAb itself (HK, GL) over an isotyped matched Ig (CAMPATH 1-g), in this particular situation"

In the reminder of the discussion, Applicants point out that the author discusses the theoretical use of mixed molecule, non-binding, antibodies in tolerance induction concluding that:

> " It may be seen that, in practice, we can never completely match the spectrum of immunogenic peptides derived from a therapeutic mAb via non-binding variants"

Applicants note that this discussion is limited solely to the use of mixed molecule variants (HK and GL). Applicants submit that it does not discuss, mention, nor even hint at the possibility of using non-mixed molecule therapeutic antibody variants. In fact, even the discussion of mixed molecule antibody variants, Applicants point out, ends on a somewhat pessimistic note, as is evidenced from the passage above. When this is taken into account, this document taken as a whole, or in combination with any of the documents dealing with humanization of antibodies, is not believed by Applicants to provide any motivation whatsoever to the skilled person to attempt to modify non-mixed molecule therapeutic antibodies to remove binding, nor does it suggest or hint that such antibody variants could be used in tolerance induction. In

addition, Applicants note that the teaching of Isaacs required the use of a two-stage tolerization process where two antibodies (HK and GL) were required to induce tolerance. The present invention overcomes this problem by providing a system which requires a sole antibody.

Reconsideration is requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE, P.C.

By Mary J. Wilson

Mary J. Wilson
Reg. No. 32,955

MJW:tat

1100 North Glebe Road
8th Floor
Arlington, Virginia 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Amend the claims as follows.

2. (Amended) An antibody as claimed in claim 25 [1], wherein the framework regions of the variable domains of the antibody have the same or substantially the same amino acid sequence as the therapeutic antibody framework regions.

6. (Twice Amended) An antibody as claimed in Claim 25 [1], wherein the CDRs are foreign with respect to the constant region of the antibody.

7. (Twice Amended) An antibody as claimed in Claim 25 [1], wherein the CDRs are foreign with respect to the heavy and light chain variable domain framework regions.

9. (Twice Amended) An antibody as claimed in Claim 25 [1], wherein the therapeutic antibody has affinity for CD52.

13. (Twice Amended) An antibody as claimed in Claim 25 [1], wherein the constant domains of the antibody have substantially the same amino acid sequence as the therapeutic antibody constant regions.

14. (Twice Amended) A fragment of an antibody according to Claim 25 [1], which fragment retains tolerance-inducing capability of the antibody.

16. (Twice Amended) An antibody or fragment as claimed in Claim 25 [1], which is monovalent.

17. (Twice Amended) An antibody or fragment as claimed in Claim 25 [1], for inducing tolerance to the therapeutic antibody in a patient.

ABSTRACT OF THE DISCLOSURE

This invention relates to an antibody which is a modified version of a therapeutic antibody with affinity for a cell-surface antigen, the antibody having reduced affinity for the antigen compared with the therapeutic antibody as a result of a modification or modifications to the antibody molecule, wherein the antibody is capable of inducing immunological tolerance to the therapeutic antibody. The invention further relates to a method of inducing immunological tolerance to a therapeutic antibody, comprising administering to a patient an antibody which is a modified version of the therapeutic antibody and which has reduced affinity for the antigen as compared with the therapeutic antibody.

D2